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Study the effects of mesenchymal stem cell conditioned medium injection in mouse model of acute colitis

Sedigheh Pouya[a,1], Maryam Heidari[a,1], Kaveh Baghaei[b], Hamid Asadzadeh Aghdaei[b], Afshin Moradi[c], Saeed Namaki[a], Mohammad Reza Zali[c], Seyed Mahmoud Hashemi[a,d,*]

[a] Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Basic and Molecular Epidemiology of Gastrointestinal Disorder Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran
[c] Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran
[d] Department of Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and aim: Inflammatory bowel disease (IBD) is an autoimmune-inflammatory disorder that results in inflammatory responses in individuals who are genetically susceptible. Uncontrolled inflammation in Crohn's disease (CD) or Ulcerative colitis (UC) affects the patient quality of life. Current therapies are not completely effective while cell therapy, especially the treatment with mesenchymal stem cells (MSCs) absorb lots of attention due to its immunomodulatory properties. So, we examined the effects of mesenchymal stem cells-conditioned medium (MSC-CM) in the experimental model of acute colitis.

Material and method: MSC-CM was isolated from C57Bl/6 male mice and stored. The acute colitis induction in C57BL/6 mice was performed by dissolving dextran sulfate sodium (DSS) in drinking water and then CM injected intraperitoneally. During the study body weight changes, bleeding, stool consistency, disease activity index (DAI), mortality rate, weight and length of the colon and histopathological analysis were recorded as well as changes in the percentage of Treg cells. The level of IL-17, IL-10, and TGF-β were measured, too. Data were reported as mean ± SD and analyzed by One-Way ANOVA test.

Results: Based on the results it is recognized CM inhibited the weight loss and bleeding and improved fecal consistency and DAI. Macroscopic examination of the colon showed that after infusion, colon inflammation was reduced and histopathological analysis showed a decrease in mucosal degeneration. The percentage of Treg cells, secretion of IL-10 and TGF-β was increased while the IL-17 level was reduced.

Conclusion: This study showed that mesenchymal stem cell secretion with immunomodulatory properties has the potential to reduce inflammatory responses.

1. Introduction

Inflammatory Bowel Disease (IBD) is a disorder with chronic and recurrent inflammation in the gastrointestinal tract which has two major forms of Ulcerative Colitis (UC) and Crohn's disease (CD) [1]. The incidence and prevalence of IBD have increased dramatically over the last few decades. So, same as Europe and America [2], the incidence and prevalence of IBD increased even in low-prevalence regions such Iran [3–6]. Although the exact cause of IBD has not been identified yet, it seems that an imbalanced immune response against the intestinal microflora is responsible for the disease in the ones with sensitive genes [7]. Existing evidence has shown both parts of the immune systems, especially T lymphocytes, are effective in the development and progression of the disease, following the activation of the innate immunity components [7–11].

The aim of different methods in IBD treatment is improving clinical symptoms and prevention of recurrence. Today, because of a primary or secondary failure of the approved therapies or sophisticated surgery for a particular patient, new and effective therapies are necessary [12–15].

The new treatments are not only have aimed to control the inflammation but also introduced to repair the damaged tissues. One of these treatments is stem cell therapy, including mesenchymal stem cells

* Corresponding author at: Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, 1985717434, Tehran, Iran.
E-mail address: smmhashemi@sbmu.ac.ir (S.M. Hashemi).
[1] Author 1and 2 contributed equally and should be considered co-first authors.

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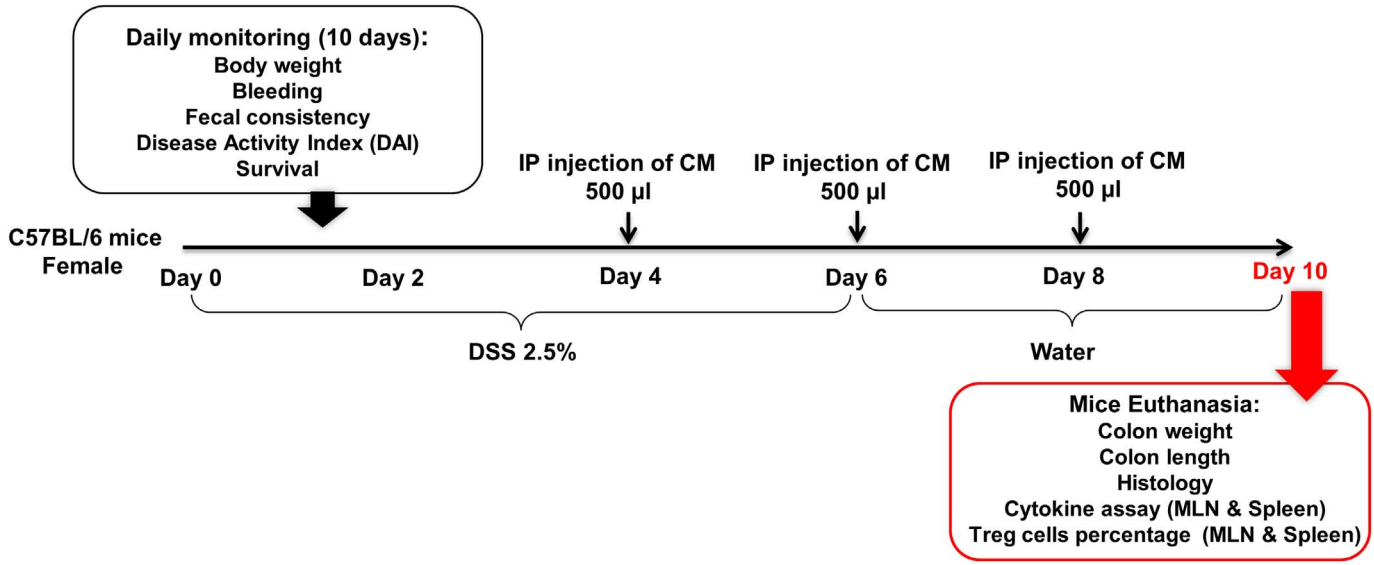

Fig. 1. Study design for DSS induced acute colitis.

[16]. These cells are able to maintain their proliferation and self-renewal ability and via production of secretome can regulate immune responses. Recently, It has been proved that these cells play their roles both cell-cell interactions and paracrine secretions including cytokines, hormones and extracellular vesicles content (containing peptides, proteins, mRNAs and micro RNAs) [17,18].

Many studies have emphasized the role of various mesenchymal stem cells mediators in the immune responses regulation [19–24]. Evidence has also shown the immunomodulatory effects of soluble factors. Direct control of $CD4^+$ T cells functions depend on several factors secreted by MSCs and the differentiation of Treg cells is achieved by secreting HLA-G5 [18,25–27].

According to previous information, T cells play an important role in the pathogenesis of IBD and MSCs are able to perform a major part of their immunomodulatory effects by secretive factors. Since cell-cell intraction is not the main tool for their inhibitory effects on lymphocytes, a probability is also considered that the paracrine activity of mesenchymal cells plays a prominent role in immune response inhibition. So considering the importance of the issue, we investigated the effects of mesenchymal stem cells- condition medium injection on the experimental model of inflammatory bowel disease.

2. Materials and methods

2.1. Mice

Female C57BL/6 mice, 6–8 week old, specific- pathogen-free were purchased from the Royan Institute of Tehran. Mice were housed 2 weeks before the experiment for adaptation. Access to adequate water and food for mice was provided and all the tests were carried out according to the approval of the Ethics Committee of the Faculty of Medicine, Shahid Beheshti University of Tehran (Code of Ethics: IR.SBMU.MSP.REC.1395.401).

2.2. Isolation and expansion of mesenchymal stem cells

C57Bl/6 mice were euthanized with using CO_2. In sterile condition, after cutting the midline of the abdominal region and isolation the adipose tissue, it was digested with type I collagenase, 0.1%. Then, homogenous tissue was centrifuged and the sediment was cultured in DMEM High Glucose medium (GIBCO) with 15% fetal bovine serum (FBS) and Penicillin-Streptomycin 0.01% in 75 cm^2 flask, at 37 °C in 95% humidity and 5% CO_2 [28]. After 2–3 weeks with 80% confluency, the cells were passaged.

2.3. Immunophenotyping of mesenchymal stem cells

Mesenchymal stem cells from the second passage were stained with Anti-CD90 (E01383-1632), Anti-CD44 (E01238-1632), Anti-CD45 (E012431635), Anti-CD73 (E01346-1632), Anti-CD11b (E01072-1632) antibodies according to the manufacturer's instructions (all from eBioscience).

2.4. Osteogenic and adipogenic differentiation potential of MSC

To evaluate the differentiation potential of mesenchymal stem cells into adipose and bone tissue, stem cells from the second passage were cultured in special culture medium for 21 days and then stained with Oil red-O and Alizarin Red-S to confirm. To induce osteogenic differentiation, MSCs were cultured in supplemented media with glycerol phosphate (10 mM), dexamethasone (100 mM), and ascorbic acid-2 phosphate (5 g/ml) for 3 weeks. For adipogenic differentiation, MSCs were cultured in complete media supplemented with indomethacin (100 mM), 3-isobutyl-methylxanthine (0.5 mM), dexamethasone (250 mM), and insulin (5 mM) for 21 days [29].

2.5. Preparation of mesenchymal stem conditioned medium

Briefly, to prepare the CM, MSCs from the second passage were cultured until 80% confluency. The medium with less FBS was then replaced and this step repeated every 2–3 days. MSCs gradually were cultured with less FBS. So, the cells sequentially were adapted to the serum-free medium and the development of toxins and unwanted proteins from oxidative stress-related changes were also prevented [29,30]. Then the supernatant was filtered using filters 0.22 µm and stored in − 70 °C. This optimal medium was used for injection into mice with acute colitis.

2.6. Colitis induction and treatment procedure

The acute colitis was induced in C57BL/6 mice that received 2.5% dextran sulfate sodium (DSS) in drinking water for 6 days and then 4 days consumption of normal water [31]. This solution was prepared daily. In order to investigate the effects of MSC-CM, 500 µl of it was injected on days 4, 6 and 8, intraperitoneally (Fig. 1).